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Anatomical changes during early gonad development in the protogynous greasy grouper *Epinephelus tauvina* (Forsskal)

GRACE MATHEW AND KURUVILLA MATHEW*
Central Marine Fisheries Research Institute, Kochi - 682 018, Kerala, India
**Cochin University of Science and Technology, Marine Science Campus, Cochin - 682 016, Kerala, India*
e-mail: gracejacob1985@yahoo.com

ABSTRACT

Anatomical changes during early gonad development of the culturable species of grouper *Epinephelus tauvina* was studied. All juveniles developed an ovarian phase initially followed by the bisexual phase gonad. Bisexual phase gonad occurred when the fish was around two years old and in the length range of 188-380 mm total length. The minimum size at first sexual maturation for females was 380 mm total length at an age of two and a half years. The presence of bisexual phase and spermatogenic cysts in juvenile gonads in *E. tauvina* could be an indication of primary male differentiation. Based on gonadal histological study of *E. tauvina* till first sexual maturation, it was found that all the juveniles developed an ovarian structure initially and subsequently developed a bisexual phase with male tissue scattered among lamellae, prior to first sexual maturation as females. Although it was not possible to find any primary males among fishes collected from the wild, the developmental significance of a bisexual phase in *E. tauvina* juveniles is assumed to be similar to diandric protogynous species, having secondary males as well as primary males developed directly from juveniles through sexual differentian.

Keywords: Anatomical changes, Diandric, *Epinephelus tauvina*, Gonad development, Greasy grouper, Monandric, Protogynous

Introduction

Groupers of the genus *Epinephelus* are widely distributed in the tropical and subtropical seas around the world. Groupers have white, tender and tasty meat that make them much relished and highly priced marine food fish. Serranids generally have characteristics favourable for culture. Many species under the genus *Epinephelus* such as *E. akaara, E. salmoides, E. tauvina, E. fuscoguttatus* and *E. malabaricus* are suitable for mariculture owing to fast growth, good feed conversion rate and high adaptability in different culture systems (Ruangpanit and Yashiro, 1995). The greasy grouper, *Epinephelus tauvina*, is an important candidate species in marine finfish culture in Asia (Chua and Teng, 1979). The sexual pattern of this species is protogynous hermaphroditism. Only limited study has been carried out on the early gonadal development of this speceis. It is still unknown whether this species is monandric (having secondary males developed from sex change of functional females) or diandric (having secondary males as well as primary males developed directly from juveniles through sexual differentiation). Diagnosis of sexual pattern is particularly challenging in groupers because differences in testicular morphology between primary and secondary male developmental pathways are not as readily apparent as they are in other fish families such as Labridae and Scaridae (Liu and Sadovy, 2004). The gonads of secondary males differ clearly from primary males, and retain the lumen from the functional female phase and develop sperm sinuses within the gonadal wall during sex-change. Species of *Epinephelus* studied to date, exhibit an ovarian structure, with a lumen and sperm sinuses within the gonadal wall. Understanding the sexual pattern in the Epinephelines requires considerable care and examination of a wide range of body sizes and ages, including both juvenile as well as adult phases employing histological techniques.

Gonads of all hermaphroditic serranids are similar in gross appearance. *Epinephelus tauvina*, a protogynous hermaphrodite, does not exhibit any externally distinguishable sexual characters. The abdomen of the female fish becomes flabby during spawning period due to the enormous increase in size of the ovaries. But ovarian and testicular tissues are not separated by connective tissue; these two tissues or female / male germ cells are intermixed during the course of sexual development. The smallest body size at female sexual maturation in *E. tauvina* is reported to be 355 mm in standard length (SL) at over two years of age (Liu and Sadovy, 2004).

The present study examines early gonad development in juveniles of *Epinephelus tauvina* until first sexual maturation, for understanding its male developmental pathway and to determine whether it is monandric or diandric.

Materials and methods

Collection , processing and gonad histology

Stages of maturity of the gonads were taken from fish samples (57 numbers), ranging in total length (Lt) from 84 to 445 mm, collected from the wild, caught in Chinese dip nets operated at Cochin bar mouth and the nearshore areas. The collected samples were immediately preserved in ice and transported to the laboratory.

Each fish was weighed to the nearest milligram, total length and standard length of fishes were measured to the nearest millimeter. The abdomen was cut open to expose the ovary and gross morphological observations of the ovary were made. Gonads were dissected out and fixed in neutral buffered formalin. The fixed gonad samples were weighed (0.001g) and transverse sections of tissues removed from anterior, middle and posterior regions for processing and analysis using standard histological techniques (Mackie, 2000). Sections were cut at 5-7 μm thickness and stained using haematoxylin and eosin (Mackie, 2000). These sections were examined under a compound microscope to determine the sex and stage of reproductive maturity of each fish. Photomicrographs of the histological preparations of the gonads were taken using a Leitz binocular microscope equipped with an automatic exposure system.

According to gonadal morphology, the gonads were classified as: ovarian phase-1 (Ov-1), ovarian phase-2 (Ov-2), bisexual phase-1 (Bi-1), bisexual phase-2 (Bi-2) and bisexual phase-3 (Bi-3). Sex and maturation stages were defined based on the most advanced developmental stage of oocytes, proportion of spermatogenic cysts, appearance of sperm sinuses and presence of sperm.

Results and discussion

The production of egg, well equipped with the necessary reserve food for the developing embryo, occurs through the processes taking place in the germ mother cells of the ovary. These include a series of events starting from activation of primordial germ cells to the differentiation of highly yolk-equipped ova. The oogonial cells transform themselves into mature ova with sufficient yolk for the development of the embryo.

Histological examination of gonads of *E. tauvina* during the present study showed the presence of two ovarian phase gonads: Ov-1 and Ov-2. Ov-1 was first observed with the formation of the ovarian lumen in early juveniles. Primary growth stage oocytes and chromatin nucleolus stage oocytes (O1) were visible and none of the oogonial cells entered meiosis. Ovarian phase gonads with ovarian lumen and oogonia in mitosis were found in juveniles in the length group 90-110 mm total length (Table 1, Fig. 1). By mitotic division of the primary oogonial cells, the secondary oogonial cells which are larger than the primary oogonial cells were formed. Gonads developed into Ov-2 with the occurrence of meiosis of O1; Ov-2 also had secondary oogonial cells together with the proliferation and growth of O1 and the development of a lamellar structure (Fig. 2).

Table 1. Size group, sample size and gonadal status of *E. tauvina* used in the study

Size group (mm)	Sample size (Nos.)	Predominant gonadal status
85-100	11	Ovarian phase-1 (Ov-1)
98-200	13	Ovarian phase-2 (Ov-2)
190-250	10	Bisexual phase-1 (Bi-1)
200- 350	12	Bisexual phase-2 (Bi-2)
340-400	11	Bisexual phase-3 (Bi-3)

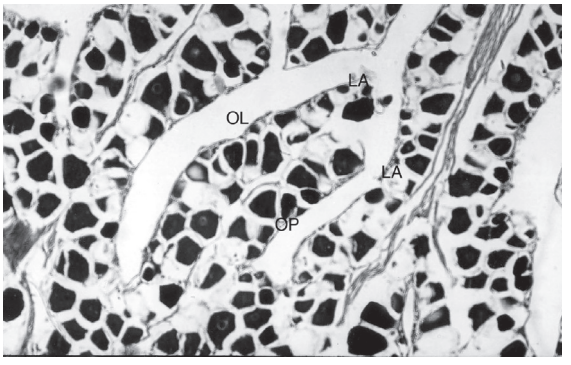

Fig. 1. Ovarian phase 1 (Ov-1) in *E.tauvina* juvenile. OG: oogonia in primary growth stage; OP : oogonia in mitosis phase; LA: ovarian lamellae; OL: ovarian lumen. (H&E; X 200)

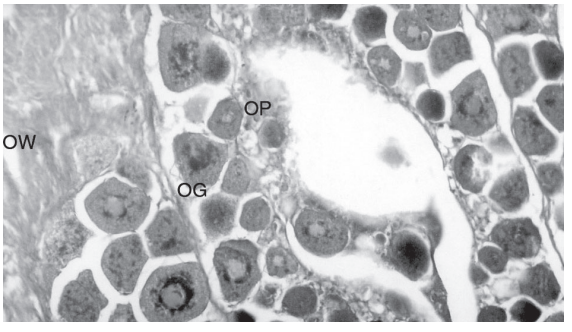

Fig. 2. Ovarian phase 2 (OV-2) in *E. tauvina* juvenile. OW: ovarian wall; OP: primary growth stage oocytes; OG: oogonia (H&E; X 200)

Three bisexual phase gonads were recognized in *E. tauvina* on further development of the Ov-2 stage. The appearance of scattered spermatogenic cysts among oogonial cells in the ovarian lamellae was first observed in Bi-1 indicating its development from ovarian phase two (Ov-2).

This was observed in juveniles in the length range of 190 to 240 mm (Fig. 3). Perinucleolar stage oocytes, early vitellogenic oocytes with multiple nucleoli arranged around the periphery of the nucleus in strands, oocytes in meiosis stage and primary growth oocytes were observed in this stage.

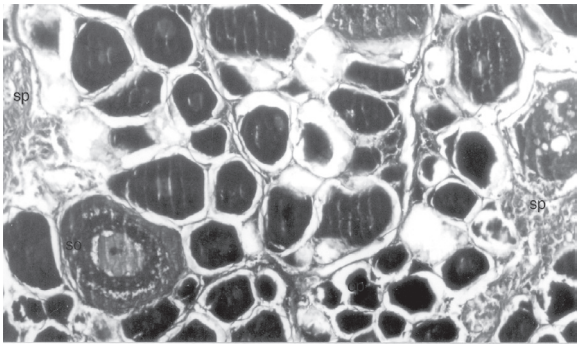

Fig. 3. Bisexual phase 1 (Bi-1) in *E.tauvina* juvenile. OP: primary growth oocytes; SO: secondary stage oocytes; SP: spermatogenic tissues. (H&E; X 200)

On further progression, oocytes in cortical alveoli stage and yolk vesicle stage were observed along with spermatogenic cysts interspersed. Bisexual phase-2 with the concomitant appearance of ovarian phase- 3 (Ov-3) and scattered spermatogenic cysts was observed prior to and also overlapping with the female maturation season, suggesting female differentiation and maturation occurring concomitant with Bi-2 phase gonads (Fig. 4).

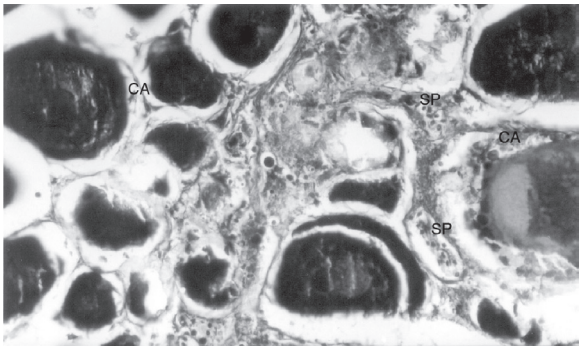

Fig. 4. Bisexual phase 2 (Bi-2) in *E.tauvina* pre-adult. CA: oocytes in cortical alveoli stage; SP: spermatogonial cysts and spermatocytes (H&E; X 200)

Around the stage of female maturation, at 340-400 mm total length and at age of 2-3 years, gonads advanced to bisexual phase-3 (Bi-3) with the concomitant appearance of vitellogenic oocytes with protein yolk globules, oocytes in cortical alveoli stage as well as ripe oocytes and also sperm sinuses, within the gonadal wall. Together with this, scattered spermatogenic cysts and proliferation of presumptive spermatogonial cysts, were also observed (Fig. 5).

In the present study based on gonadal histology of *E. tauvina* untill first sexual maturation, it was found that all juveniles developed first an ovarian structure which then developed into a bisexual phase (male tissue scattered in lamellae) prior to first sexual maturation as females. Although we have not found any primary males in the

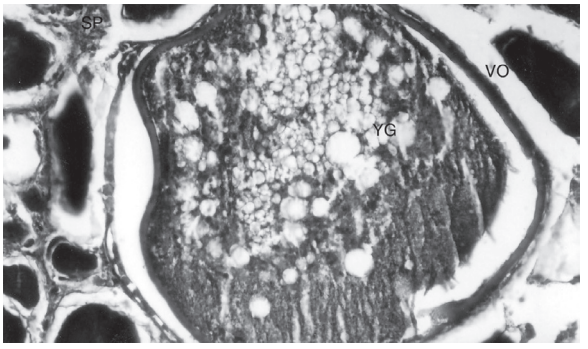

Fig. 5. Bisexual phase 3 (Bi-3) in *E.tauvina.* VO: vitellogenic oocytes; YG: protein yolk globules; SP: spermatogonial cysts and spermatocytes (H&E; X 400)

samples collected, the developmental significance of a bisexual phase in *E.tauvina* juveniles is assumed to be similar to a closely related diandric protogynous species *Cephalopholis boenak* (Liu and Sadovy, 2004). With further development of different stages of spermatogenic cysts, and proliferation of presumptive spermatogonial cells, this could suggest a male differentiation pathway (*i.e.,* primary male differentiation since it develops directly from the juvenile phase). If primary males exist in *E. tauvina*, we can assume that their gonadal development is from ovarian phase to bisexual phase-1 (Bi-1) or bisexual-phase-2 (Bi-2) to bisexual-phase-4 (Bi-4) and then to testis as in *C. boenak*.

The present study confirms that all juveniles in this species pass through an ovarian phase and then a bisexual phase prior to first sexual maturation as females, although it was not possible to confirm whether it is monandric or diandric, since primary males have not been found during sampling from the wild. In other words, the gonadal developmental paths of females are known from ovarian phases to bisexual phase-1 (Bi-1) to bisexual phase-2 (Bi-2) to bisexual phase-3 (Bi-3). Functional females may change sex to secondary males *i.e.,* from Bi-3 to testicular phase (T). In the present study in *E. tauvina,* gonad differentiation was first observed in the 88 to 92 mm L_T group. Unlike in the gonochoristic species, gonad development during sexual differentiation has not been studied much in protogynous groupers. However, Murata *et al.* (2009) observe that in the Malabar grouper, *Epinephelines malabaricus,* presence of testicular tissue was not found during gonad differentiation and that there were no primary males in *E. malabaricus* and all males developed from females by sex change.

Bisexual phase gonads with scattered spermatogenic cysts and with ovarian structure have been reported in juveniles of a few epinephelines (Smith, 1965; Siau,1994; Adams, 2003). The developmental significance of bisexual phase-1 was also proposed as the gonadal phase that all juveniles passed through prior to sexual differentiation in bi-directional sex change. More rapid sex change could be obtained due to at least in part, the common presence of non-functional sperm tissue in ovaries and overlap in proliferation of male tissue and degeneration of female tissue in transitional gonads (Mackie, 2000; 2003). In protogynous coral trout, *Plectropomus* sp., Adams (2003), observed that the sperm sinuses which occurred within the ovaries become functional only after sex change and this was considered to be a mechanism to minimize non-reproductive time and to maximize flexibility in male development.

In general, the developmental changes observed in the gonad of *E. tauvina* in the present study is similar to the *Epinephelus* type (Smiths, 1965). Although it was not possible to find any primary males in the wild, the development of a bisexual phase in *E. tauvina* juveniles is assumed to be significant which indicated its sexual plasticity, *i.e.,* primary male development and male-to-female sex change. The gonads of teleosts and cyclostomes develop from single primordia directly in the peritoneal epithelium underlying the genital ridge and corresponds only to the cortex (Brusle-Sicard *et al*., 1992; 1994; Guraya, 2000). It has been suggested that this may account for the more widespread occurrence of intersexuality among cyclostomes and teleosts (Hoar and Randall, 1969).

Juvenile sex differentiation can be controlled by social factors in protogynous grouper and could be applied in broodstock management in grouper breeding by obtaining males through social control instead of resorting to hormone manipulation (Mackie, 2003). The presence or absence of a larger male plays an important role in adult female sex change; female(s) do not change sex in the presence of a larger male, but change sex after the removal of the larger males in the same social group.

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